

CHAPTER I

Introduction

1.1 Overview

Cyanide ion is a perilous chemical that is harmful to humans and animals. It enters the body by inhalation, ingestion, and skin absorption. Once inside the body, it inhibits the oxygen transfer process in the cytochrome [1], causing toxicity. Cyanide ion is released by industrial processes such as in pesticides production, metal coating by electrolysis, and X-ray film recovery, etc [2]. In addition, cyanide is found in natural sources such as bamboo shoots, cassava, and barley. Bacteria and fungi also produce cyanide compounds which is the most common source of naturally-derived cyanide contamination in the environment [3]. Because of its toxicity, the World Health Organization (WHO) mandated that the highest concentration of cyanide contamination allowed in food and drinking water is 2.7 ppb [4]. Detection of cyanide ion under biologically relevant aqueous solution is vital for health and safety as well as quality control in many industries. To determine the presence of cyanide ion in water, two main methods are employed. The first method is colorimetric whereby the concentration is ascertained using spectrophotometric measurement and color comparison [5]. The second method uses Amperometric titration to determine cyanide concentration. In this method, AgNO_3 is used as the titrant. When AgNO_3 reacts with CN^- , it is converted to $\text{Ag}(\text{CN})_2^-$ and the end point is observed by both visual and amperometric methods [6]. Both of these methods are time consuming, require expensive instrumentation and has a relatively low sensitivity. Therefore, in this work we intend to develop a novel method for quantitative detection of cyanide ion using fluorescence spectrometry. This method be highly sensitive is expected to low cost and more rapid than the conventional methods.

1.2 Structures of fluorescence compounds

Recently, conjugated molecules or polymers have emerged as one of the most important classes of signal transducing materials which can be used as a sensing element in fluorescent sensor. They transform a chemical signal to easily measurable electrical or optical readout. Fluorescence based methodologies have attracted much interest due to their intrinsic sensitivity and selectivity [7]. Considerable efforts have been devoted to the design of fluorescent compounds to be used as transducers. The

molecules with delocalized π -electron systems have attracted a considerable interest due to their versatile optical and electrical properties [8].

A selection of well-known conjugated polymers with fluorescent capabilities are shown in **Figure 1.1**. They are, polythiophene (1) [9], polypyrrole (2), [10], polyfluorene (3) [11], poly(*para*-phenylenevinylene) (4) [12], and poly(*para*-phenyleneethynylene) (5) [13]. The delocalized electronic structure of the π -conjugated system enables them to exhibit efficient absorption and strong emission, and produce amplified signal changes upon interacting with various analytes.

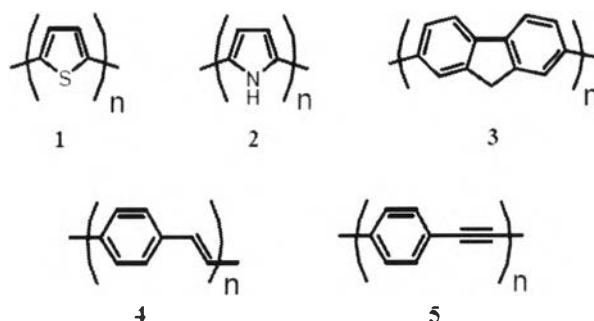


Figure 1.1 Molecular structures of some common π -conjugated polymers

Another class of compounds that can be classified as fluorophores are the π -conjugated small molecules. Shown in **Figure 1.2** are examples of popular commercial fluorescent dyes [14]. The structure of these compounds has been designed to occupy specific location in the fluorescent excitation/emission spectra.

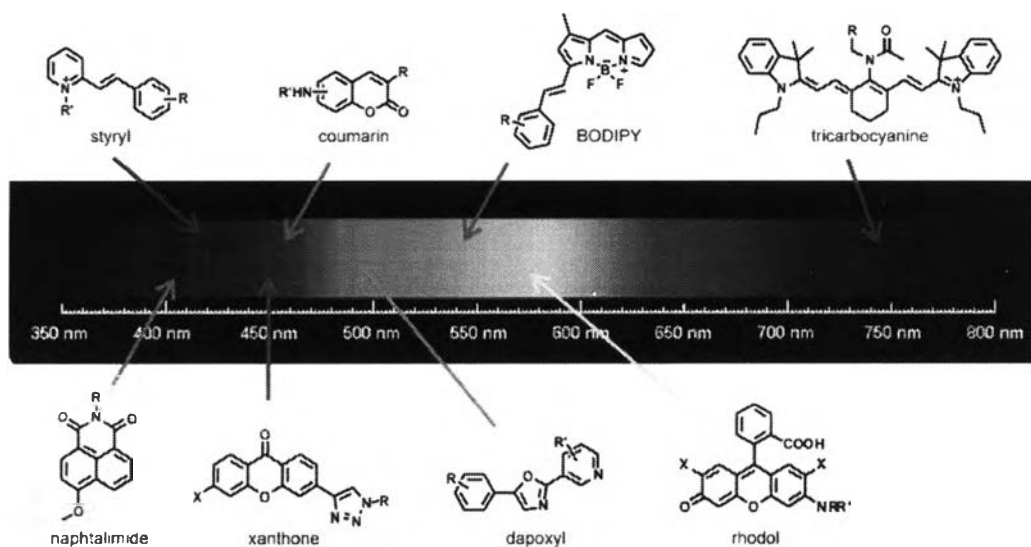


Figure 1.2 Molecule structure of some common π -conjugate molecules and their emission range in the visible region.

1.3 Introduction to fluorescence

Fluorescence is the emission of light by π -conjugated molecule that has absorbed UV or visible light. Induction of molecules in the ground state (S_0) by light energy causes the electrons to be excited to higher electronic state called excited state (S_1) or higher while maintaining the same geometry based on the principle of Franck-Condon (**Figure 1.3**). Subsequently, substances will undergo internal conversion whereby the electron emits energy via non-radioactive decay and returns to S_1 level. This is the state with the most stable geometry. It is also called a locally excited (LE) state. Finally, fluorescent emission would occur and the substance returns to its ground state.



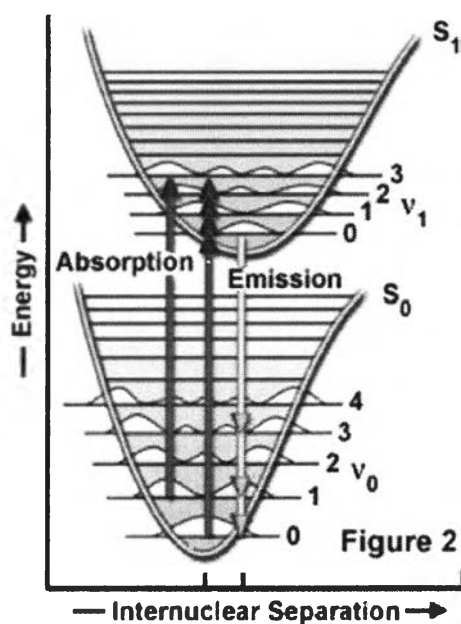


Figure 1.3 Franck-Condon energy diagram explaining fluorescence process. [15, 16]

A reduction in fluorescence intensity is referred to as quenching. This is caused by several mechanisms such as Photo-induced Electron Transfer (PET), Förster Resonance Energy Transfer (FRET), and Internal Charge Transfer (ICT). In this research work, we focus on the Internal Charge Transfer (ICT) technique.

1.4 Internal charge transfer

In some cases, molecule in the LE state may undergo other pathway to release the energy without emission (non-radiative decay). For example, a compound with the structure composed of both good electron donating and withdrawing groups of which an electron pair can delocalize via the π -conjugated system which is called internal charge transfer (ICT) process to convert to the ICT excited state (Figure 1.4). This ICT state has lower energy with different geometry from the LE state. ICT excited state then relax to the original ground state that has the same geometry as ICT excited state. This relaxation may either give light within or outside the visible light spectrum [17, 18]. Due to multi-step process, ICT emission generally has lower fluorescence quantum yield comparing with LE state. The ICT state usually gives longer wavelength and is usually stabilized by polar solvent (Figure 1.4) due to ICT excited state stabilization [19, 20]

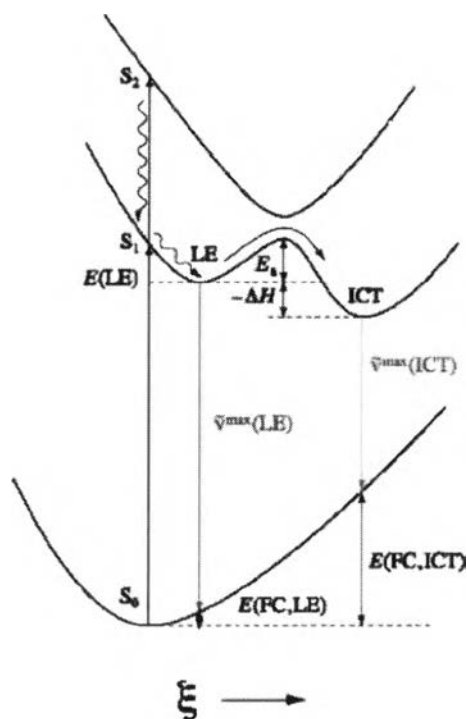


Figure 1.4 Potential energy surface of the ground state (S_0) is excited to S_1 or S_2 and then relaxed to LE state, and ICT state (FC = Franck Condon).

1.5 Literature review on fluorescence sensor for cyanide ion

Due to nucleophilic properties of cyanide ion, most cyanide ion sensors are designed to incorporate electron deficient groups to enable addition reaction of cyanide ions (**Figure 1.5**). For example, Badugu co-worker used boronic acid derivative **1r** to generate a turn on mode fluorescence sensor showing a detection limit ca. around $20 \mu\text{M}$ [21]. Moreover, they also developed a boronic acid derivative **2r** to probe cyanide ions in aqueous media [22]. The latter possesses a detection limit of around $50 \mu\text{M}$. Recently, heterocyclic ammonium derivatives (**3r**) has been used as a detector for cyanide ions in aqueous media with $0.5 \mu\text{M}$ detection limit (**Figure 1.5, 3r**) [23]. In addition, Lee et al. have discovered that salicylaldehyde derivatives (**4r**) can be used as a selective probe for cyanide ion in mixed solvent containing 40% DMSO in HEPES buffer media with $10 \mu\text{M}$ detection limit [24].

The fluorescence enhancement mechanism of a compound containing salicylaldehyde derivatives **4r** is shown in **Scheme 1.1** [24]. Briefly, the receptor in **4r** comprises of hydroxyl as an electron donating group and aldehyde as an electron withdrawing group. Their presence causes an internal charge transfer that leads to a low fluorescence signal. Addition of hydride leads to the formation of anion **4r-2** as confirmed by NMR titration experiment. This inhibits internal charge transfer and enhances fluorescence emission (**Figure 1.6**).

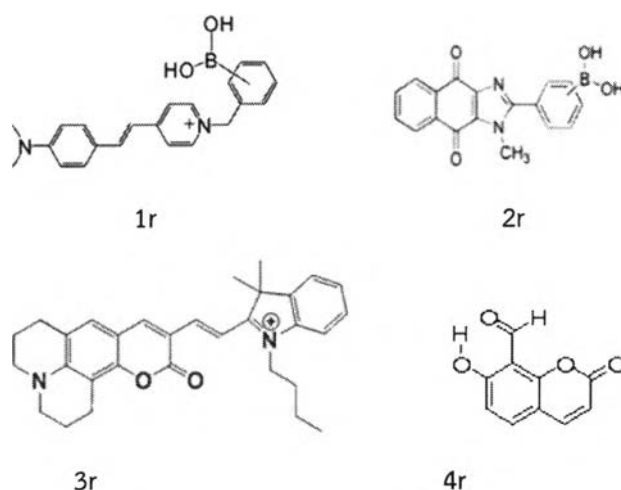
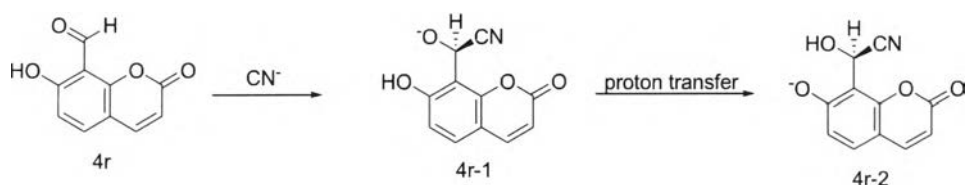


Figure 1.5 Fluorescence probe for the detection of cyanide ion



Scheme 1.1 The mechanism of cyanide ion turn on of coumarin derivative **4r**.

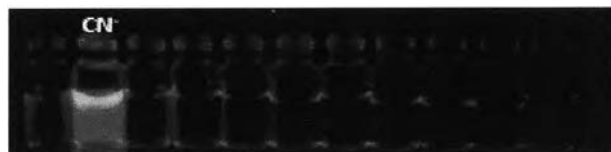
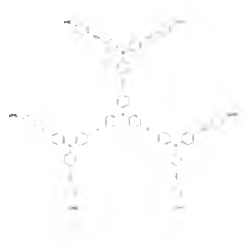
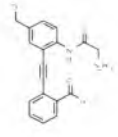
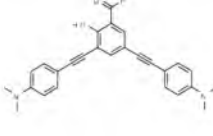


Figure 1.6 Color changes of **4r** solutions upon addition of cyanide ion and other anions under visible light.

Beside salicylaldehyde group, others have used aldehyde group as a receptor for turn on mode fluorescence sensor. A dendrimer **5r** composed of three aldehyde sensory units demonstrates high sensitivity and selectivity towards specific amino acid such as cysteine or homocysteine in DMSO media [25]. The system shows fluorescence enhancement ratio ca. 30 in the presence of amino acid at 0.3 mM concentration. Similarly, diphenylene acetylene derivative containing amide and aldehyde group (**6r**) also demonstrates high sensitivity and selectivity towards specific amino acid [26]. It undergoes fluorescence enhancement upon exposure to cyanide ion in aqueous media. The detection limit of this fluorophore is 2.5 μM . Recently, our group reported the cyanide sensor fluorophore **7r** having diphenyl acetylene as a fluorophore and salicylaldehyde as a receptor [27]. In HEPES buffer pH 7.4, our system showed excellent fluorescence signal when exposed to cyanide ion. The detection limit of this fluorophore **7r** is 2.5 μM .

Table 1.1 Example of fluorescence sensor for amino acid and cyanide ion.

Fluorophore	Mode	Analyte	LOD
 5r	on	Cysteine	NA
 6r	on	CN ⁻	2.5 μM
 7r	on	CN ⁻	2.5 μM

1.6 Poly(phenylene ethynylene)s and their sensor applications

Polyphenylene ethynylene (PPE) is a class of conjugate polymer which possesses ethynyl and phenyl group linkage in its polymer back bone as shown in (Figure 1.7). Since it possesses a long conjugated back bone, it has a great photo-physical properties such as high fluorescence quantum efficiency [28] and good thermal properties. These properties make PPE suitable as semiconducting polymer, photo luminescence material, and optoelectronic material [29, 30].

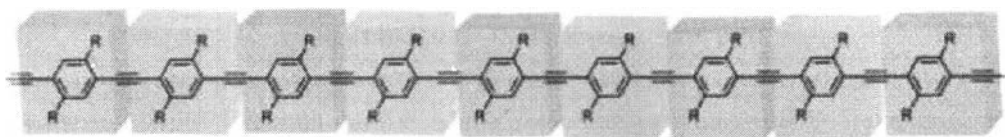
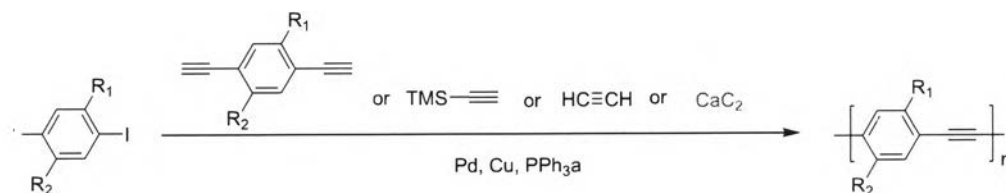


Figure 1.7 Structural model of poly(phenylene ethynylene)

In general, PPEs are synthesized via Pd-catalyst coupling between diiodobenzene and alkyne as presented in Scheme 1. The alkyne coupling partner can be classified into four classes such as diyne, protected acetylene, acetylene gas and calcium carbide [28]. The first two classes use diethynylarenes and protected acetylenes as a C-C triple bond source. Although the starting materials are easy to handle because most of them are in solid or liquid form, however, this method possess several disadvantages such as a requirement for multiple steps synthesis and high cost of its starting materials. The third option uses acetylene gas as a starting material. Although it is cheap, it is difficult to handle because it is in a flammable gaseous form that is difficult to set up.

The latest method developed by Fery-Forgues, S. is the synthesis of poly(phenyleneethynylene) using calcium carbide as an acetylene source. This method demonstrates significant advantage above all methods mentioned above [31, 32]. It provides an easily manageable process, customized for routine laboratory work that utilizes a cheap and naturally available starting material [33-35].



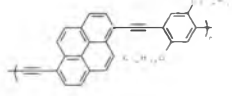
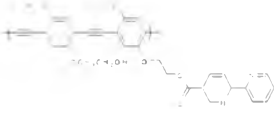

Scheme 1.2 Synthesis route of poly(phenylene ethynylene)s

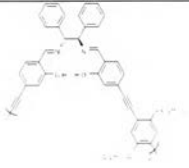

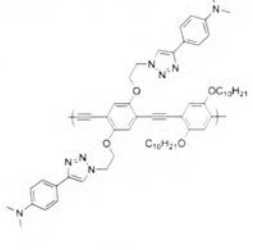
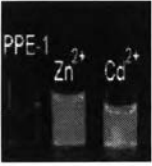
Based on the process previously developed by our group, in this work we plan to use calcium carbide as a starting material in the preparation of PPEs-based fluorescence sensor for cyanide ion detection.

1.7 Literature review on sensor from poly(phenylene ethynylene)s

Poly(phenylene ethynylene)s (PPE) is not only convenient to prepare, it also has good optical properties and high quantum efficiency. Thus, it becomes a popular fluorophore for sensor application [36]. Structurally, the PPE sensor always contains a receptor probe attached to a pendent position on a polymer side chain as shown in the in **Table 1.2**. The PPE **8r** contain pyrene as a receptor unit displays fluorescence quenching in the presence of explosive compound TNT in gas phase [37]. The **9r** polymer which has bipyridyl group can be used to detect mercury ion [38]. Moreover polymer **10r** which has hydroxylamine group shows specific sensing ability to Zn^{2+} in a fluorescence turn on mode [39]. Base on above examples, most of PPE sensor were used as turn off mode due to the strong fluorescence nature of PPE. Recently, the turn on PPE sensor were first report by Pourghaz in 2011, demonstrating the PPE attached to triazole unit undergoing a strong fluorescence enhancement in response to several cation such as Zn^{2+} and Cd^{2+} ions [23]

Table 1.2 Example of fluorescence sensor form PPE.

Fluorophore	Analyte	mode	Color	LOD
 8r	TNT	off	-	-
 9r	Hg^{2+}	off		-

 <p style="text-align: center;">10r</p>	Zn^{2+}	on		-
 <p style="text-align: center;">11r</p>	$\text{Cd}^{2+}, \text{Zn}^{2+}$	on		3mM

Lastly, the **12r** polymers with hydroxylamine as a probe can be used to detect several anions such as α -hydroxyl carboxylic acid and L- isomer derivatives which is turn on mode [40]. The system shows fluorescence enhancement ratio ca. 8.41 in the presence of amino acid at 1 mM concentration.

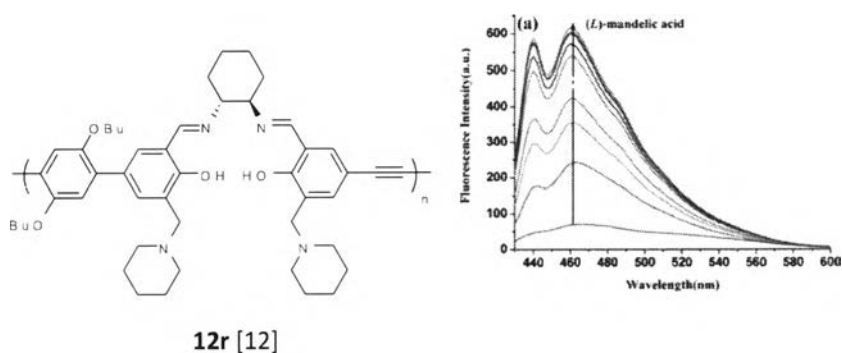


Figure 1.8 Structure of PPE containing hydroxylamine (left) and fluorescence enhancement for α -hydroxyl carboxylic acid and L- isomer derivatives of **12r** (right).

Previous researches mentioned above use PPEs as a fluorophore in various sensing applications. They rely on fluorescence turn off mode and are broadly used

for metal or natural analyte detection. Therefore, it remains a challenge to design and prepare a PPE sensor for anion detection as turn on mode fluorescence sensor.

We hypothesize that if we prepare a PPE polymer containing anion receptor having ICT properties, we should achieve a low emissive PPE with enhanced fluorescence response to anions, especially cyanide ions (Figure 1.7).

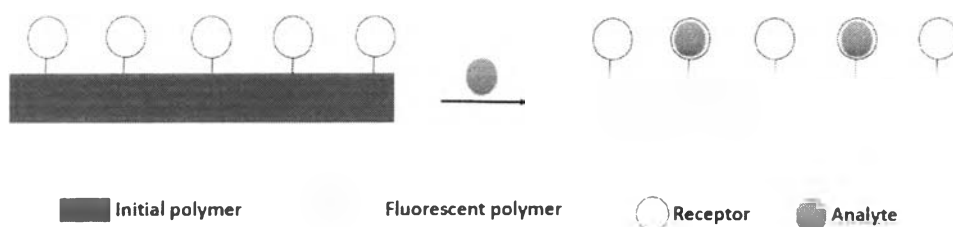
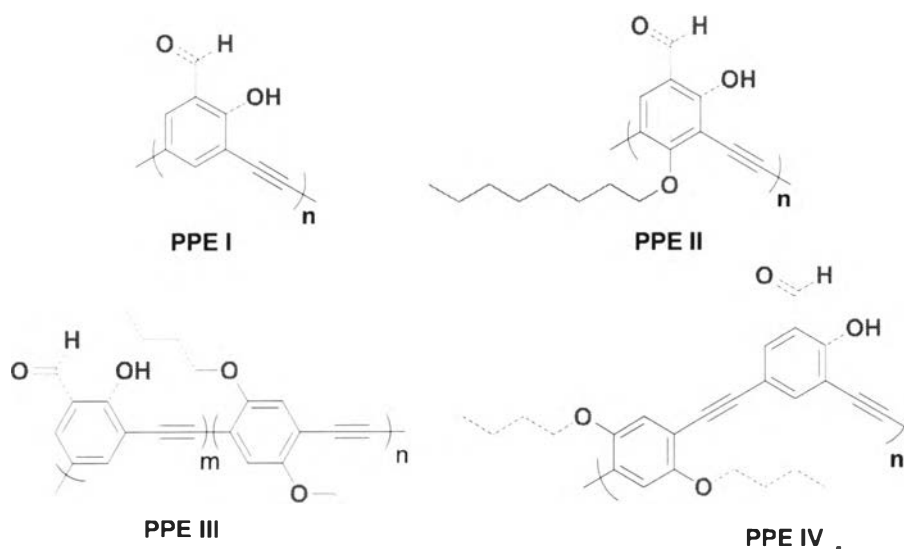


Figure 1.9 Fluorescence sensor model for the detection of cyanide ion.

1.8 Objective of this work

In this research work, we are interested the synthesis of poly(phenylene ethynylene) PPE I, PPE II, PPE III, and PPE IV containing salicylaldehyde group as a receptor unit. All the prepared PPEs will be tested their sensing ability with various anions. This work would allow us to create the first fluorescence turn on sensor using conjugate polymer for the detection of cyanide anion.



Furthermore, small molecules of PE I and PE II will be synthesized and investigate their sensing ability toward a panel of anions in order to compare the sensing properties.

